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NUTRITION REVIEWS

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NUTRITION REVIEWS

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FATAL ACUTE MYOCARDIAL INFARCTION: SEX, RACE, DIABETES AND OTHER FACTORS

Acute myocardial infarction is one of the most common causes of death in the United States and every conceivable approach (experimental, clinical, anatomical and epidemiological) is being utilized in an effort to dispel the mystery that surrounds it. As part of the wide approach to the study of the disease, Drs. Wilbur A. Thomas, Kyu Taik Lee, Robert M. O'Neal and Erwin R. Rabin, pathologists at Washington University in St. Louis, have recently determined the incidence of acute myocardial infarction in several large series of autopsies that were performed between 1910 and 1954. Some of the results of their studies have been reported (*Arch. Int. Med.* **97,** *421 (1956)*; *Ibid.* **98,** *80 (1956)*; *Ibid.* **98,** *489 (1956)*; *Arch. Path.* **60,** *616 (1955)*; *Am. Heart J.* **52,** *581 (1956)*) and other results will be reported in subsequent articles.

Every autopsy from Barnes Hospital was classified by age, sex, race and period of death (1910 to 1939 or 1940 to 1954) and the incidence of acute myocardial infarction was determined for each decade of life, each sex, each race and each period. The data obtained were correlated with information regarding the incidence of diabetes mellitus, obesity and hypertension in the various groups. Similar, though more limited, studies were made of the autopsy series at Homer G. Phillips Hospital in St. Louis, Charity Hospital in New Orleans, and Howard University Hospital in Washington. An acute myocardial infarct as defined in these studies indicates histologically recognizable death of cardiac muscle of less than one month's duration. The duration was determined as accurately as possible by the clinical history and anatomical findings. In most instances the myocardial infarct was due to occlusion of an atherosclerotic coronary artery by a thrombus.

Investigators in the past have almost invariably found a higher overall incidence of myocardial infarction among White men than among White women (*see* P. D. White, *Heart Disease*, Macmillan, N w York (1951)). In studies based on clinical diagnoses the relative incidence in the two sexes have varied from 3:1 to 7:1 (males:females). Very little information is available from autopsy series because most investigators reporting data from the latter source have simply stated the number of patients of each sex with coronary arteriosclerosis and its complications and have not determined the ratio of the sexes in the entire autopsy population. It obviously is necessary to know the sex distribution in the population being analyzed before one can determine the relative incidence of a disease in the two sexes. One cannot assume that the sexes will be evenly divided in a large autopsy series. Most series that have been analyzed, including the series at Barnes Hospital, contain considerably more men than women. However, data regarding the relative incidence of myocardial infarction are available from at least one other large autopsy series covering the period 1910 to 1938. Among 30,265 autopsies performed at the University of Minnesota during the period of 1910 to 1938, Clawson (*Am. Heart J.* **22,** *607 (1941)*) found the ratio of the incidence of deaths from coronary arteriosclerosis to be 2.5 males to 1 female.

The most remarkable data obtained from the Barnes autopsy series are those pertaining to changes in the relative sex incidence of acute myocardial infarction. In the period 1910 to 1940, the incidence of acute myocardial infarction in the Barnes autopsy series was 2 males to 1 female. This difference in incidence in the two sexes is much less than that in most studies based on clinical diagnoses but is quite similar to the ratio of

2.5 to 1 reported by Clawson in the Minnesota autopsy series for the years 1910 to 1938. Thus, prior to 1940, data from several different sources indicated that the overall incidence of coronary arteriosclerosis and its complications was greater in men than in women. When the period 1940 to 1954 was studied by the group at Washington University it became apparent that a startling change had occurred. In the Barnes autopsy series for the period 1940 to 1954, the ratio of the incidence of acute myocardial infarction in the two sexes approaches 1:1 (actually 1.1 male to 1 female, which is not a significant difference in this series). After the completion of the study of the Barnes autopsy series, similar data were obtained regarding recent periods from two other autopsy series. Achor, Burchell and Edwards (unpublished data quoted by Lee and Thomas in *Arch. Int. Med.* **98,** *80 (1956)*) found a similar ratio of incidence of acute myocardial infarction (1:1) between the sexes among patients autopsied at the Mayo Clinic during the period 1946 to 1950. The group at Washington University was allowed to examine a large sample of the autopsy series at Charity Hospital in New Orleans and they found in the period 1940 to 1954 that the incidence of acute myocardial infarction was actually higher in White women than in White men. (However, the difference was not statistically significant so that it can be considered as 1:1).

There seems no reason to doubt that prior to 1940 the ratio of the incidence of coronary arteriosclerosis and acute myocardial infarction among White individuals was actually at least 2 males to 1 female. Evidence from all sources, including the Barnes autopsy series, supports this conclusion. Data based on at least 12,000 autopsies on White adults from three centers (St. Louis, New Orleans, Rochester) indicate that since 1940 the ratio of incidence of acute myocardial infarction in the two sexes is 1:1. Information is needed from numerous other sources before definite conclusions are drawn. However, in view of the large sample that already has been analyzed, it seems unlikely that further studies of autopsies will alter the results significantly.

According to all classical studies the incidence of acute myocardial infarction is far greater among young White men than among young White women and this disproportion decreases with increasing age and finally disappears. The relative incidence in the two sexes in the Barnes autopsy series follows this general pattern and differs only in degree. In both periods (1910 to 1939 and 1940 to 1954) the incidence of acute myocardial infarction was significantly greater in men than in women in the younger age-groups. Among the older age-groups in the period 1910 to 1939, the incidence in the two sexes was similar. However, among the older age-groups in the period 1940 to 1954 the incidence of acute myocardial infarction was higher in women than in men. Thus in the recent period the incidence was higher in young men than in young women but higher in old women than in old men and the resulting overall ratio was 1:1.

The entire autopsy series was divided into age-groups for each sex (40 to 49, 50 to 59, and so on) and the incidence of acute myocardial infarction was determined in each age-group. Since the percentage incidence of acute myocardial infarction among old women was much greater in the period 1940 to 1954 than in the preceding period, it is apparent that the changing sex ratio is due, at least in part, to a disproportionate rise in the incidence of acute myocardial infarction among old women and not simply to either an absolute or relative increase in the number of old women in the autopsy series.

The relative incidence of acute myocardial infarction in the two sexes among autopsied White individuals in New Orleans was similar to that in the corresponding age-groups at Barnes Hospital. Information related to this feature is not yet available from the autopsy series at the Mayo Clinic.

No evidence was found to indicate that the changing sex ratio of acute myocardial infarction could be accounted for by a changing incidence of diabetes mellitus, hypertension, or obesity. However, it should be pointed out that in both periods (1910 to 1939 and 1940 to 1954) the relative incidence of acute myocardial infarction in the two sexes was different among diabetic patients than among non-diabetic patients (among diabetic patients before 1940 the sex ratio for acute myocardial infarction was 1:1, but after 1940 there was a preponderance of women; among non-diabetic patients before 1940 the sex ratio was 2.3 women to 1 man and after 1940 it was 1.4:1). Although a change has occurred in the relative sex incidence among diabetic patients and non-diabetic patients, acute myocardial infarction is still slightly more common among male non-diabetic patients than among female non-diabetic patients and it is slightly more common among female diabetic patients than among male diabetic patients.

Most studies in the United States and Africa have indicated that the incidence of acute myocardial infarction is lower among Negroes than among Whites. The pathologists at Washington University wanted to determine whether or not changes in incidence had occurred among Negroes similar to those changes among Whites. Since the autopsy series from Barnes Hospital consists largely of White individuals it was necessary to examine others. The Negro components of autopsy series from 4 hospitals were analyzed with the cooperation of Drs. J. O. Blache, R. S. Jason, R. L. Holman, and H. C. McGill, Jr. These hospitals were Barnes, Charity of Louisiana in New Orleans, Howard University in Washington D. C., and Homer G. Phillips in St. Louis. Approximately 8,000 autopsies performed on adult Negroes between 1910 and 1954 were analyzed and compared with approximately 10,000 autopsies performed on adult Whites from St. Louis and New Orleans.

The overall incidence of acute myocardial infarction was 5 times as high among the Whites as among Negroes. The difference was much greater in the period 1940 to 1954 than in the period 1910 to 1939 because the incidence among White individuals had risen tremendously but that among Negroes had risen only slightly. The difference in incidence increased with increasing age because the incidence rose sharply among Whites with increasing age but showed little change among Negroes. The increasing difference in the incidence of acute myocardial infarction with increasing age is not simply a matter of difference in longevity, because the data on incidence refer to the percentage incidence among those who survive to a given age. For example, more than 25% of a group of White women who reached the age of 70 died of myocardial infarction and the corresponding figure for a group of Negro women over 70 years old was only 2%. The incidence of acute myocardial infarction among Negroes was similar in the two sexes in both periods that were studied.

Information from all available sources indicates a rising incidence of acute myocardial infarction among White individuals in the United States and the data from the autopsies studied by the group at Washington University tend to confirm this rise. The overall incidence of acute myocardial infarction among the Barnes autopsies was 29 times as high in the decade 1945 to 1954 as it was in the decade 1910 to 1919. The rise occurred in all age-groups of both sexes and was not simply a result of an ageing population. No evidence was found to indicate that the rise in incidence could be accounted for by a rise in the incidence of diabetes mellitus, hypertension, and obesity. It seems likely that some changing factor(s) in our civilization is responsible and it is remarkable that its effect has been almost exclusively confined to members of the White race.

Many investigators have noted a close association between diabetes mellitus and coronary heart disease. The Washington

University group found that acute myocardial infarction was 4 times more common among autopsied White diabetic patients than among autopsied White non-diabetic patients. However, among Negro diabetic patients, acute myocardial infarction was not significantly more common than among non-diabetic patients. Thus a Negro diabetic patient is even less likely to die of myocardial infarction than a non-diabetic White patient. It is interesting to note that diabetic patients with acute myocardial infarction did not die at an earlier age than non-diabetic patients.

Many observers have thought that coronary heart disease was more common among overweight individuals than among those of normal weight or less. However, some recent reports have appeared suggesting that this relationship has not been proved, at least in certain age-groups. S. M. Garn, M. M. Gertler, S. A. Levine, and P. D. White (*Ann. Int. Med.* **34,** *1416 (1951)*) found no connection between body weight and myocardial infarction in young men. Terminal weights were available for most of the Barnes autopsies in all age-groups. Several comparative groups were established but no connection between the terminal body weight and the incidence of acute myocardial infarction was demonstrated in any age-group. Furthermore, no evidence was found to indicate that weight loss had been a prominent feature in the patients with acute myocardial infarction.

Most investigators have postulated a close association between coronary heart disease and hypertension. However, some recent workers including L. H. Sigler (*Ann. Int. Med.* **42,** *369 (1955)*) have suggested that the association may be merely the chance association of 2 common diseases. The Washington University group used the average weight of the kidneys of the patients with acute myocardial infarction as a rough guide to the degree of hypertension during life. The average weight of the kidneys of the patients with acute myocardial infarction was less than that among patients with "normal" kidneys and more than that among patients that died directly from hypertension. However, there was no significant difference in the average weight of kidneys of patients who died of cancer and those who died of acute myocardial infarction. Thus, with this crude test, the investigators at Washington University were unable to demonstrate any greater association between acute myocardial infarction and hypertension than between carcinoma and hypertension.

The data obtained from autopsy series by the Washington University group confirm some widely held views regarding acute myocardial infarction but do not confirm others. The facts regarding the incidence of acute myocardial infarction among 17,000 autopsies that are the most interesting are: (1) the discovery that a change has occurred in the relative incidence of the disease in White individuals of the two sexes from 2:1 (males:females) before 1940 to 1:1 since 1940; (2) the discovery that the incidence in older White women has increased more since 1940 than in other age-sex-groups; (3) the discovery that the difference between the incidence in Negroes and Whites is increasing rather than decreasing; (4) the discovery that diabetes mellitus did not have a significant effect on the incidence in Negroes although diabetes mellitus unquestionably has a profound effect on the incidence of acute myocardial infarction among White individuals.

Many other studies must be made before the full significance of these data are known. The Washington University pathologists state that even in their own hospital the clinical diagnosis of acute myocardial infarction was made more often in men than women during the same period in which the incidence was 1:1 in the two sexes among autopsies. It seems imperative that clinical material be carefully re-evaluated before final conclusions are drawn. Another aspect that must be explored further is the effect of race. The differences in the incidence of acute myocardial infarction among Negroes

and Whites should not be attributed to genetic factors until every other possibility (particularly possible differences in dietary habits) has been exhausted.

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ELECTROLYTE METABOLISM IN KWASHIORKOR

The syndrome of kwashiorkor is under active investigation in many parts of the world and from many points of view (*Nutrition Reviews* **13,** *67* (*1955*)). Among the most prominent abnormalities present in this disease are the gross disturbances in water, electrolyte, and protein metabolism manifest clinically in nearly every case by edema and hypoalbuminemia. An investigation of the electrolyte disturbance in patients having kwashiorkor, and its relationship to nitrogen balance, has recently appeared (J. D. L. Hansen, *South African J. of Lab. and Clin. Med.* **2,** *206* (*1956*)).

Seven males with edema, hypoalbuminemia, a history of a post-weaning diet, and with recent diarrhea, were studied by a balance technique including separate collection of urine and feces. The first 2 patients were treated with diets consisting of milk with a supplement of potassium acetate. Cumulative balances, which are presented in detail in the study, showed an initial small retention of sodium and a gain in weight, followed by a considerable negative balance accompanying the loss of edema. Potassium and nitrogen were retained throughout the experiment, with maximal uptake of potassium in the first 3 days at a potassium to nitrogen diet ratio that greatly exceeded 3 mEq per gram. Following this initial period the ratio between potassium and nitrogen uptake approximated 3 mEq per gram (the ratio of these elements in muscle). It was noted that there was considerable diuresis and loss of edema before a significant rise in the serum proteins took place.

The subsequent 5 cases were placed on nitrogen-free diets for four to seven days to investigate the effect on the edema of supplying only electrolytes and carbohydrates. In these cases potassium retention was maximal in the first two or three days, at a time when the infants were consistently in negative nitrogen balance. Following introduction of nitrogen into the diet, potassium retention paralleled that of nitrogen. Actual retentions of potassium on the nitrogen-free regimens varied between 3.18 and 9.9 mEq per kilogram of body weight, a degree of depletion approximating that seen in severe infant diarrhea.

The initial period of sodium retention was of variable duration, being as long as six to eight days in 2 cases. The sodium diuresis was not necessarily dependent upon the protein intake, although in 2 cases the sodium diuresis and loss of edema occurred only following the introduction of milk feedings. In one case there was a suggestion that a positive potassium balance was essential for the continuation of the sodium diuresis. Nitrogen was retained by all of the patients in amounts somewhat larger than the retentions in 2 well-nourished control patients of the same ages. The sodium, potassium, and chloride concentrations in the serum were measured at intervals during the studies. The pre-treatment sodium and chloride levels showed a wide scatter within the accepted normal range with little, if any, shift during treatment. The pre-treatment concentrations of serum potassium were significantly depressed and rose to normal levels in conjunction with potassium repletion.

These balance studies are interpreted by Hansen to suggest an absolute and relative potassium deficiency in these cases. The

mechanism of this potassium depletion was probably the diarrhea, the fecal loss of potassium being rather large in some of the studies. This was accompanied by a relatively low potassium intake due partly to anorexia and partly to the low protein content of the diet. Accurate information concerning the electrolyte content of the diets fed these infants before hospitalization was not available. Several possible endocrine dysfunctions were discussed which might play a role in the development of edema under these conditions. The absence of hychloremic alkalosis in the face of potassium deficiency of this magnitude may well be accounted for by the lack of sodium loading in these patients, as pointed out by F. D. Moore (*Metabolism* **4,** *379 (1955)*; *Nutrition Reviews* **14,** *3 (1956)*).

This clear-cut demonstration of potassium deficiency of major proportions in these infants with kwashiorkor, and the retention of potassium and occasional alleviation of the edema on a nitrogen-free regimen, would suggest that the electrolyte imbalance was closely related to the development of edema. This also might explain discrepancies between the severity of the edema and the level of plasma proteins which occasionally occurs. Furthermore, this study suggests that, in the initial treatment of kwashiorkor, the high potassium concentration of skim-milk may be fully as important as its protein content.

NUTRITIONAL STATE FOLLOWING SURGERY

The influence of removal of the stomach, or of other surgical procedures, on the nutrition of the patient depends upon the extent of the resulting surgical trauma as well as upon the dispensability of the functions which are lost following surgery. The loss of weight following surgical procedures is believed to be the result of increased catabolism of body protein concomitant with an increased adrenal cortical hyperactivity. A. J. Paquin and J. Lange, (*Ann. Surg.* **144,** *809 (1956)*) have studied postoperative body weight loss in a diversified group of surgical patients in order to observe the effect of sex, degree of trauma, postoperative feeding, and basal preoperative weight on the change in body weight during the immediate postoperative period. The intensity of surgical trauma was classified as minimal, moderate, or severe, depending on the amount of damage to tissue and the duration of the operation. Minimal trauma was exemplified by procedures such as local biopsy, severe trauma included such operations as radical removal of visceral cancer, and any process of intermediate intensity was classed as moderate trauma.

In general, the duration of weight loss was directly proportional to the intensity of trauma. The weight loss in the minimal group stopped after two days. In the moderate group the loss continued from one to fourteen days and in the severe group the period of loss ranged between six and twenty-eight days. The rate of weight loss in the minimal and moderate groups was unpredictable, but in the severe group if weight loss continued longer than five days the rate was more uniform, approximating 4.5 grams per day per kilogram of basal preoperative weight. As a result, the percentage of body weight lost was proportional to the duration of the loss. In spite of changing from parenteral to oral feeding, all patients continued to lose weight. Although heavier patients tended to lose weight longer than lighter patients, accurate prediction of the rate or duration of loss could not be made on the basis of the preoperative weight or the postoperative feeding regimen. Although the number of cases was too small for an accurate assessment of the differences, it appeared that when operative trauma was of nearly the same intensity women lost weight

at a faster rate, but for a shorter period, than did men. The percentage of weight lost was less for women than for men, but the women ate adequate diets sooner than did men.

The stomach acts mechanically as a reservoir for temporary food storage and slow release of this to the intestine, as well as chemically for the continuation of digestion and release of specific metabolites such as the intrinsic factor. A. H. Moreno (*Ann. Surg.* **144,** *779 (1956)*) has described the operations that have been used to cure gastric cancer and has reported the effects of some of these operations upon the nutritional disturbances of a series of patients. Because of the high mortality rate in the early history of total gastrectomy (complete removal of the stomach) it has been customary to employ a partial or subtotal gastrectomy for most cases of resectable gastric cancer. Recently the total removal has become more popular because the mortality rates have decreased but evidence that the results have improved is lacking, especially in the face of the nutritional impairment and other disturbances which accompany the complete removal of the stomach.

By varying the type of anastomosis (the surgically formed passage between organs) or by producing substitute gastric pouches, attempts have been made to decrease the amount of disability resulting from total gastrectomy. The study being reviewed was initiated because of conflicting opinions regarding the effects of this surgical procedure, and because the effects which may be created influence the selection of the surgical procedure. No consideration of the curative merits was reported.

Ten patients with gastric cancer were studied in follow-up periods of varying length after undergoing different types of operations. The results of these studies were compared with those of metabolic and other studies done on 10 normal subjects. The small intestine, comprising in sequence the duodenum, the jejunum, and the ileum, is sufficiently long so that it may be used to construct a substitute pouch without materially decreasing its secretory and absorptive efficiency. After removal of the stomach, a loop of jejunum was raised and attached to the esophagus at the apex, and then separated from the rest of the jejunum, which was reunited. In the near-total and subtotal gastrectomies, the stomach remnant was joined to the jejunum. This was done by excluding the duodenum but without disturbing the normal sequence of the other parts of the intestine.

Several types of information were recorded in the comparison between the normal control subjects and the surgical patients. The body weight before and after surgery was compared with the average weight over five years before the onset of the disease, and also with the ideal weight. Metabolic studies were performed over three- to four-day periods in which a diet of constant composition was fed. Fat and protein assimilation were determined, glucose tolerance tests were done, and gross and microscopic examinations of feces were made. Complete studies of the blood were recorded. The filling and emptying time of the remnant stomach or substitute reservoir was determined by X-ray studies done at intervals following ingestion of barium-coated meals.

All patients lost weight both before and after surgery, the postoperative loss being stabilized after thirty days which is a period similar to that occurring in other major surgical procedures reviewed above. The total gastrectomy groups lost about 5.5 kilograms while the near-total group averaged a loss of 7 kilograms. The weight recovery period, though variable, averaged 4 kilograms for the total gastrectomy group in which the esophagus was joined to the jejunum and 7.8 kilograms for the total gastrectomy group having a jejunal reservoir. The subtotal and near-total group gained an average of 5.3 kilograms. More fecal fat was excreted after surgery than normally, and the total gastrectomy groups

excreted more than the others regardless of the presence or absence of a pouch. No significant differences in fecal nitrogen loss were apparent in the variously operated groups.

Glucose tolerance tests indicated rapid absorption and reactive hypoglycemia but no intergroup differences. Microscopic examination of feces showed abnormal amounts of undigested materials but no differences in the groups. Determination of the mean diameter of the red cells in all gastrectomized patients showed macrocytosis (the presence of abnormally large erythrocytes) without anemia. X-ray studies revealed that the substitute pouches did serve to retard the passage of food and that emptying time was very close to that of a normal stomach. Patients undergoing partial resection had smaller food capacities and a more rapid evacuation. Transit time studies indicated that patients with total gastrectomy had increased activity in the upper jejunum and compensatory decreased movement in the ileum. The presence of the substitute pouch allowed small amounts of food to be presented to this more active region.

Since the different operative procedures left the patients with differing food capacities, the amount of food that could be ingested without discomfort was variable. In the groups with no remnant of stomach or substitute reservoir, it was necessary to eat 6 or 7 meals daily in order to approach a normal intake. In spite of this expediency, maintenance of normal weight and recovery of lost weight was not achieved in this group. In addition, the loss of nutriments in the stool played a minor, yet significant, role in weight loss. The author ascribed the abnormal losses of fat seen in these various types of operations to "inadequate biliary and pancreatic secretions, defective mixing of food with the digestive juices, increased intestinal motility, and lack of the intermittent evacuation of a gastric reservoir." Although pernicious anemia develops in patients with complete removal of the stomach, presumably due to lack of the antianemic intrinsic factor, this usually occurs from two to five years after the operation.

There was no evidence in this study to explain the origin of the macrocytosis observed. Symptoms of distress, and feeling too full after meals, were typical of the group in which total gastrectomy was followed by joining the esophagus to the jejunum. These patients did not return to their usual activities as readily as the others, became easily fatigued, and felt generally weak. The use of a substitute reservoir improved weight gain, food capacity, symptoms after meals, and the ability to return to a normal life, although it failed to correct abnormalities in fat, carbohydrate, and nitrogen metabolism. Since the remnant of stomach left after near-total or subtotal gastrectomy was able to enlarge after a time and assume a more nearly normal function, this would seem to be the preferred type of operation. The decision regarding the type of repair to be employed should be postponed, however, until the main objective of complete removal of the malignant lesion has been fulfilled.

ESTROGENS AND CARCASS COMPOSITION OF BEEF CATTLE

Approximately ⅔ of the beef cattle raised in this country are now receiving estrogens, either orally or by implantation during the fattening period. This practice is an outgrowth of earlier discoveries that estrogen treatment of cattle resulted in faster body weight gains and improved efficiency of feed utilization by some mechanism not entirely understood (*Nutrition Reviews* **13,** *110, 333 (1955)*; H. Welch and F. Marti-Ibañez, editors, "Symposium on Medicated Feeds", *Medical Encyclopedia, Inc., New York*

(*1956*)). Several recent studies contribute additional information on the effect of estrogen administration on carcass characteristics.

R. J. Deans, W. J. Van Arsdell, E. P. Reineke, and L. J. Bratzler (*J. Animal Sci.* **15,** *1020* (*1956*)), in studies on carcass composition and grading of steers, have compared the effects of feeding diethylstilbestrol (an artificial estrogen) and the effects of implanting a combination of progesterone and estradiol with untreated control steers. A total of 42 steers were used and fed a practical ration. As in all the studies, estrogen treatment increased body weight gains. No significant differences from the two treatments were found in dressing percentage, carcass grade, or in the size of the adrenal glands, heart, liver, spleen, or hide. As estimated from physical characteristics of rib samples, carcasses from diethylstilbestrol-fed animals showed no changes in proportions of lean meat, fat, or bone. However, a significant lowering of fat and an increase in lean meat was produced by the implantation of progesterone and estradiol which was confirmed by chemical analysis. There was no significant differences between the water content of various meat samples or of external or internal fats in the several groups studied.

W. M. Beeson, F. N. Andrews, M. Stob, and T. W. Perry (*J. Animal Sci.* **15,** *679* (*1956*)) have studied the effect of feeding diethystilbestrol on the carcass composition of 36 steers. A 12 per cent increase in growth was obtained but the results these researchers obtained on carcass composition were somewhat different from the previously mentioned study. They reported that estrogen-fed steers had a significantly smaller dressing percentage (58.7 per cent as compared with 60.0 per cent) and slightly inferior carcass grades as compared with untreated control steers at the end of the 179-day experiment. Chemical analysis of meat from representative rib samples of 18 animals indicated that estrogen-fed steers had significant increases in water (from 42.5 to 47.4 per cent) and protein (from 12.3 to 14.2 per cent) content of the meat and a significant decrease in the fat content (from 44.8 to 38.3 per cent).

Contrary to the above results, J. Kastelic, P. Homeyer, and E. A. Kline (*J. Animal Sci.* **15,** *689* (*1956*)) found that, although growth was improved, no differences in carcass characteristics could be attributed to the feeding of diethylstilbestrol in a study with 92 steers. Measurements were made of carcass grades and the fat, lean, and bone content of representative tissue samples. The variations between individual animals within the various groups was greater than the variation between groups. At least part of the explanation for these differences in conclusions of various laboratories may be that the different rations used either have an unexplained effect on body composition or contain endogenous estrogens. It is well known, for example, that alfalfa and certain other feedstuffs such as clover, wheat, soybean meal, and oats may contain significant amounts of estrogenic activity (P. J. S. Pieterse and F. N. Andrews, *Ibid.* **15,** *25* (*1956*)).

The preceding studies have been made with diethylstilbestrol given orally. The effects of estrogen implantation on body composition as reported by different laboratories have been fairly consistent as summarized below. M. T. Clegg and F. D. Carroll (*J. Animal Sci.* **15,** *37* (*1956*)), in a continuation of their earlier studies (*Nutrition Reviews* **13,** *110* (*1955*)), used 32 steers in 2 experiments to study the effect of estrogens on carcass composition. Also, in a third experiment 28 heifers were divided into 4 groups, consisting of animals which had been ovariectomized and their control groups with and without diethylstilbestrol treatments. In all cases of estrogen treatment, 60 mg. of diethylstilbestrol were given by ear implantation which resulted in the usual weight increases.

It was found that the percentage of fat, as

determined by specific gravity measurements, was less and the percentage of lean meat greater in all estrogen-treated animals. Estrogen treatment did not affect the percentage of bone or water of the steers but caused a significant increase in the water content of the heifers (associated with the increase in lean meat). Levels of plasma glucose, non-protein nitrogen, serum protein-bound iodine, potassium, and sodium were unaffected by estrogen treatment. There was a significant increase in the cross-sectional area of the rib-eye muscle in all trials. Carcass grades were lowered in some cases. In heifers but not in steers, dressing percentage was reduced as a result of treatment. There was no effect of ovariectomy on carcass grades, dressing percentages, daily gains, or carcass composition.

The results suggested to the authors that diethylstilbestrol increased protein anabolism. The additional weight obtained in the treated steers or heifers could not be accounted for on the basis of increased water retention or upon bone development but was chiefly due to increases in lean meat.

J. N. Aitken and J. A. Crichton (*Brit. J. Nutrition* **10,** *220* (*1956*)) studied 5 pairs of identical twin steers fed a winter fattening ration. One member of each twin was implanted with 120 mg. of hexestrol (dihydrodiethylstilbestrol) and the other member used as a control. All treated animals made substantially greater gains in weight and showed better feed efficiency. No differences were obtained in carcass grades or in dressing percentages. From chemical analysis of a sample rib-cut taken from just one pair of twins and by means of regression equations, it was estimated that the carcass of the treated steer (the carcass weighing a total of 780 pounds) contained 9.5 pounds more bone, 6.7 pounds more protein, 28 pounds more water and 3.4 pounds less fat than the carcass of the untreated steer, which weighed 737 pounds. The data on carcass composition must be considered tentative since only one pair of animals was represented; however this information agrees with similar data obtained in other studies.

C. C. O'Mary, E. P. Warren, T. J. Davis, and H. H. Pierce, Jr. (*J. Animal Sci.* **15,** *52* (*1956*)) have reported also that ear implantation of diethylstilbestrol gave no differences in dressing percentages or carcass grades in a study of 50 steers. No data were given on body composition in this study.

Previous studies have shown that bulls react differently than steers to implantation of diethylstilbestrol (*Nutrition Reviews* **13,** *333* (*1956*); E. W. Klosterman, V. R. Cahill, L. E. Kunkle, and A. L. Moxon, *J. Animal Sci.* **14,** *1050* (*1955*)). Recently, this same group of workers (V. R. Cahill *et al.*, *Ibid.* **15,** *701* (*1956*)) in further studies on this subject have compared the effect of diethylstilbestrol implantation in bulls and steers on carcass composition, weight of certain endocrine glands, and palatability of meat. Using a total of 40 cattle they found, in agreement with others, that ear-implantation of diethylstilbestrol in steers caused a small reduction in the thickness of the fat layer under the skin and a small increase in lean meat as determined by physical measurements. In untreated bulls there was significantly less fat and more edible meat than in either of the steer groups (treated or untreated). Diethylstilbestrol implantation caused a slight reduction in the percentage of lean meat in the bull and a small increase in fat deposition.

The changes in fat distribution in all animals in the studies of Cahill and coworkers affected the carcass grades as measured by federal standards. The best grades were in the untreated steers ("average choice"), whereas the carcasses from treated steers were poorer ("low choice"). The bulls were graded "average-good", whereas the treated bulls were graded as "high-good". The ratio of dressed weight to body weight for all 4 groups did not vary significantly. A study of changes in endocrine glands showed that treatment of either bulls or steers resulted in heavier pituitary and adrenal glands. Thyroid gland size was re-

duced in the treated bulls but not in the treated steers. Unfortunately, in these studies no description is given of the ration fed the cattle. It is to be expected that differences in diet would affect body characteristics and it would seem important to give a careful description of the diet in reports of this nature.

Cahill and co-workers found no difference in palatability of meat from estrogen-treated animals. Similar conclusions on palatability have been made by Marion Simone, F. Carroll, E. Hinreiner, and M. T. Clegg (*Food Research* **20,** *521* (*1955*)) and E. Wierbicki, L. E. Kunkle, V. R. Cahill, and F. E. Deatherage (*Food Technology* **10,** *80* (*1956*)).

All groups of workers cited agree with earlier conclusions that daily gains were increased by estrogen administration. Good agreement also exists in the finding that implantation of estrogens resulted in somewhat less fat and more lean meat in the edible portions of the carcass. This was not as evident, however, when diethylstilbestrol was given orally since no changes in fat content were noted by J. Kastelic and coworkers (*loc. cit.*) or by R. J. Deans and coworkers (*loc. cit.*). It is difficult to see why these differences exist between oral and implanted estrogens unless it is a matter of level of estrogen available to the tissues.

In general, except for the studies of W. M. Beeson and co-workers (*loc. cit.*), no significant differences were found in dressing percentages due to estrogen treatment of steers in the 7 studies mentioned. Not as good agreement existed on the effect of estrogens on carcass grade. In 4 out of 7 studies reviewed there was a slight decrease in carcass grades as measured by federal standards (based on fat distribution and "marbling"). In the other 3 studies no differences were noted. Since several trials have shown that the tenderness of the meat was not affected by the reduction in fat, the practical significance of the grading system might be questioned.

The above studies confirm the commercial advantage in increasing the weight of steers by estrogen administration. In addition they are important in providing information which will aid in elucidating the basic mechanism by which estrogens stimulate weight gains in cattle. The variation in results obtained suggest that the strain of animals used has an influence on the observed effects. The ration and the environment may also affect the results. For these reasons, more studies providing complete information on the above points will be necessary before final conclusions can be reached in all aspects of the problem.

APLASTIC ANEMIA FROM TRICHLOROETHYLENE-EXTRACTED FEED

Food or feed processing often involves chemical treatment. Generally, if the chemical agent used is known to be hazardous, it is assumed that the level of the residue of the agent detectable in the food or feed represents an appraisal of its influence on the nutritive value of the treated substances. In some instances, however, analysis for residue does not represent a reliable approach to whether or not a given chemical treatment has altered nutritive value.

A case in point is the fatal aplastic anemia in cattle resulting from the feeding of trichloroethylene-extracted soybean oil meal (W. R. Pritchard, C. E. Rehfeld, and J. H. Sautter, *J. Am. Vet. Med. Assn.* **121,** *1* (*1952*)).

It is assumed that the anemia-inducing agent found in trichloroethylene-extract-soybean oil meal is a reaction product of some constituent in the meal with the solvent. The chemical nature of the reaction product and the mechanism of its biological effect, however, are not yet known.

Experiments are underway at the Uni-

versity of Minnesota, St. Paul, to investigate more fully the clinical, hematological, and pathological aspects of intoxication with trichloroethylene-extracted soybean oil meal. A recent paper deals with the production of the disease in the young bovine (W. R. Pritchard, *et al.*, *Am. J. Vet. Res.* **17,** *425 (1956)*). Calves weighing 250 to 300 pounds were fed alfalfa hay free-choice and a supplement composed of the following: 0.75 parts corn and cob meal, 0.75 parts crushed oats, 3.0 parts soybean oil meal, and 0.1 part mineral mixture.

Fatal acute aplastic anemia was produced in calves receiving the supplement in which the soybean oil meal had been extracted with trichloroethylene. No symptoms were observed in calves fed similar meals extracted with hexane. It was further established that (1) the severity of the disease was related to the amount of the toxic feed consumed; (2) all batches of soybean oil meal extracted with trichloroethylene did not have the same toxicity; and (3) detailed hematological studies provided the best criteria for diagnosis of intoxication.

The first blood changes noticed, on the twenty-fourth day of feeding the processed meals, was a reduction in thrombocytes. This was followed by a rapidly progressing reduction in all of the blood cellular elements and in death within 35 to 55 days. Prolonged blood-clotting time occurred in all animals in which marked reduction in blood cellular elements had occurred.

Animals developed the plastic anemia whether they were fed 1- or 3-pound amounts of the toxic meal daily. The only difference noted was the rate at which blood changes occurred, with the animals which consumed the larger amounts succumbing earlier.

Treated meals that had been established as containing relatively low amounts of the toxic substance were fed continuously for extended periods (W. R. Pritchard, *et al.*, *Ibid.* **17,** *430 (1956)*).

Animals fed 3 pounds daily of such meals developed chronic leukopenia and thrombocytopenia in 40 to 50 days. This condition persisted until the animals were slaughtered after 27 months of the experimental feeding. Three of 4 animals with the chronic blood dyscrasia were bred and produced calves. There was no evidence that the calves had been adversely affected by placental transfer of the toxic factor in the meal.

Intermittent feeding of the toxic meals resulted in the characteristic blood dyscrasia which usually disappeared during intervening periods when hexane-extracted meal replaced the toxic meal in the ration.

As already stated, various specimens of soybean oil meal treated with trichloroethylene were not uniform in their toxicity. Meals processed within a few months after the soybean was harvested consistently resulted in high concentration of toxic products after extraction with trichloroethylene. Meals extracted 10 to 14 months after soybean harvest were uniformly found to be relatively low in the toxic agent. In connection with these studies it was also observed that with the onset of peripheral blood changes there was progressive hypoplasia of the bone marrow involving all hematopoietic elements.

Using the young dairy calf as an assay animal, it was established that feeding trichloroethylene itself did not result in any of the toxic manifestations characteristic of the condition resulting from the extraction of soybean oil meal with this solvent (W. R. Pritchard, W. E. Mattson, J. H. Sautter, and M. O. Schultze, *Ibid.* **17,** *437 (1956)*).

Trichloroethylene that had been recycled for several days in a commercial extraction plant likewise was non-toxic, dispelling the theory that decomposition products of the solvent might be causative agents in the production of aplastic anemia.

Whether or not the toxic products might also be encountered in extractives from trichloroethylene-extracted soybeans (i.e., in the oil or the phospholipids which, in commercial operation, are encountered in the settlings of the extracted oil and re-

ferred to as "foots") was also determined. Feeding such extractives did not produce any of the characteristic toxicity symptoms.

It was further noted that rumen digestion of the toxic meal was not a necessary prerequisite for manifestation of toxicity. Calves fed the toxic meal through an abomasal fistula developed typical acute aplastic anemia.

The possibility of the disease representing an allergic response was also investigated. It was assumed that if the disease were an allergic reaction the calf could be sensitized to the toxic agent by feeding a high level for a short period. Later challenging with a similar quantity should then induce typical blood changes. Using this approach, no evidence was obtained to support the concept that the aplastic anemia was induced through an allergic response.

The susceptibility of sheep to this toxicity was also investigated (W. R. Pritchard, *et al.*, *Ibid.* **17,** *446* (*1956*)).

Although the blood changes characteristic of aplastic anemia were noted in some sheep fed the toxic meal, others exhibited blood changes more characteristic of hemolytic anemia. In the latter, anemia was a fairly constant finding, but the thrombocytes and total leukocytes were either in the normal range or were increased. Sheep generally appeared to be more resistant to the disease than cattle.

Two horses were fed a meal that had been established as highly toxic to cattle. Both animals developed typical blood changes and fatal aplastic anemia (W. R. Pritchard, R. Hammer, J. H. Sautter, and M. O. Schultze, *Ibid.* **17,** *441* (*1956*)). Susceptibility to intoxication thus does not appear to be restricted to ruminants.

Laboratory animals were also fed the toxic meals (W. R. Pritchard, *et al.*, *Ibid.* **17,** *448* (*1956*)). Long periods of feeding to mice, rats, hamsters, guinea pigs, rabbits, and dogs failed to reveal any evidence of aplastic anemia or other blood dyscrasia. Both guinea pigs and dogs, however, exhibited a syndrome of gradual generalized debilitation which terminated in death when the meals were fed at levels that were toxic to cattle. Feeding hexane-extracted meal did not produce this syndrome.

In order to explore the possibility of mammary gland transfer of the toxic agent in trichloroethylene-extracted soybean oil meal, 3 heavily lactating cows were fed toxic meal. The cows developed blood changes and clinical symptoms of aplastic anemia. Cats, dogs, and calves fed milk produced by these cows as the major diet component failed to show any signs of toxicity (W. R. Pritchard, W. E. Mattson, J. H. Sautter, and M. O. Schultze, *Ibid.* **17,** *444* (*1956*)).

These extensive investigations clearly demonstrate that the hematopoietic tissues of various mammalian species are quite variable in their response to the toxic agent or agents formed when soybean oil meal is extracted with trichloroethylene. It will be of interest to learn of the chemical nature of the toxic factor or factors involved.

LIPOIC ACID IN ANIMAL NUTRITION

Lipoic acid (thioctic acid, protogen, 6,8-dithiooctanoic acid) is known to be an essential growth factor for certain microorganisms and an important coenzyme in various metabolic reactions (*Nutrition Reviews* **7,** *207* (*1949*); **8,** *293* (*1950*); **11,** *59*, *360* (*1953*)).

The only available evidence for growth-promoting activity of lipoic acid in diets of higher animals is contained in a report by Betty G. DeBusk and R. J. Williams (*Arch. Biochem. Biophys.* **55,** *587* (*1955*)) who used both rats and chicks. These animals were fed purified diets consisting of sucrose, alcohol-extracted casein, gelatin, corn oil, salts, methionine, and supplements of all

vitamins (although no mention was made of the inclusion of vitamin K). Extra arginine and cystine were added to the diet fed to chicks. Lipoic acid was added at 4 different levels ranging from 10 micrograms to 10 mg. per kilogram of diet in both the rat and the chick experiments.

The addition of 1 mg. of lipoic acid to 1 kg. of the basal diet increased the weight gain of rats from 67 g. for the basal group (12 rats per group) to 96 g. for a twenty-one day period. When 10 mg. of lipoic acid were fed, the rats gained only 68 g., the same as the group receiving no lipoic acid. No explanation is offered for the apparent toxic effects of the 10 mg. level, nor was the experiment repeated.

In the studies with chicks (20 White Leghorn chicks per group) lipoic acid increased the weight gain from 72 g. for the basal group to 105 g. for the group receiving 10 mg. of lipoic acid per kilogram of diet over a twelve day period. Intermediate levels of lipoic acid gave graded responses.

In both the chick and rat studies, feed efficiency improved when weight improved. The authors (*loc. cit.*) mentioned that "enhanced growth or food utilization, or both" was obtained when other groups of chicks on 2 practical relations were given 1 mg. of lipoic acid per kilogram of diet, although no data were presented. It is difficult to understand why the addition of lipoic acid to a practical ration should give a response in growth or feed efficiency. Lipoic acid has been reported to be present in several natural materials (alfalfa, liver, yeast, etc.) and would be expected to be present in a natural diet (*Nutrition Reviews* **7,** *207* (*1949*)); (G. W. Kidder, *Fed. Proc.* **13,** *695* (*1954*)).

Contrary to the above studies, reports from 4 different laboratories indicate that lipoic acid feeding did not result in improved growth of chicks or rats. E. L. R. Stokstad, H. P. Broquist, and E. L. Patterson (*Fed. Proc.* **12,** *430* (*1953*)) were unable to obtain evidence of a lipoic acid deficiency in either rats or chicks on purified diets containing alcohol-extracted casein. More recent work from this laboratory (E. L. R. Stokstad, E. L. Patterson, A. M. Albrecht, and R. H. White-Stevens, *Proc. Soc. Exp. Biol. Med.* **92,** *88* (*1956*)), made with a crossbred strain of chicks fed both purified and practical rations, supports their earlier conclusions concerning the inactivity of lipoic acid. The authors also state that they did not obtain a growth response from lipoic acid when it was added to a sample of the same diet used by DeBusk and Williams and obtained from them. As further evidence for the lack of requirement for exogenous lipoic acid, Stokstad and co-workers presented data showing that the developing chick embryo is able to synthesize this compound. A total of 0.5 microgram of lipoic acid per egg was found in unincubated eggs, whereas a total of 11.5 micrograms was found after twenty-one days of incubation.

W. C. Supplee, G. F. Combs, and G. L. Romoser (*Arch. Biochem. Biophys.* **61,** *140* (*1956*)) also have failed to obtain growth responses with lipoic acid in chicks. They used 3 different strains of crossbred chicks fed purified diets containing either casein and gelatin or isolated soybean protein. Results were also negative when lipoic acid was added to a practical ration.

A. B. Morrison and L. C. Norris (*Poultry Sci.* **35,** *739* (*1956*)) were unable to find any growth-promoting activity for lipoic acid in crossbred chicks when they used a diet as similar as possible to the diet used by DeBusk and Williams (*loc. cit.*). Because of the possibility that the chicks showing no growth response to lipoic acid might have an intestinal flora which synthesized this compound, Morrison and Norris added 1 per cent of sulfasuxidine to the basal ration. Even under these circumstances, the addition of lipoic acid had no influence on growth or feed efficiency.

B. L. Reid *et al.* (*Proc. Eleventh Distillers Feed Conference, Cincinnati, Ohio, p. 68, March 7, 1956*) conducted 5 experiments with White Leghorn chicks which showed that lipoic acid failed to stimulate growth when injected into chicks receiving a purified diet. No experiments were reported in which lipoic acid was given in the diet.

It would appear that these differences between the results of DeBusk and Williams (*loc. cit.*) and the results of the other laboratories cannot be explained on the basis of differences in diet or in species of chicks. Until more convincing evidence is obtained one must conclude that lipoic acid is not an essential growth factor for higher animals.

PROTEIN FROM LEAVES

For a number of years there has been an interest in the possibility of using grasses and other leafy materials as a staple source of protein for non-ruminants such as pigs, poultry and, in emergency, man. Attention has therefore been focused on processes in which the protein of the leaf might be separated from the fibrous fractions, and on the evaluation of the products. The most sustained support for this work has been in Britain where the climate is unsuitable for the production of soyabean and other high-protein oil-seeds (with a resulting heavy importation of these materials as well as of animal products) while home farming can give leafy crops with yields of 400 lb. of crude protein per acre.

Experimental machinery has been operated for a number of years to express a juice from green crops, by one means or another, and then coagulate it to yield a product which, upon drying, has 30 to 60 per cent of crude protein and less than 5 per cent of crude fibre (N. W. Pirie, *Chem. Ind.* (*London*) 442 (1953)). Feeding results reported so far have been disappointing.

A further nutritional evaluation of a large number of these leaf-protein concentrates prepared from a series of different raw materials, has been reported by S. J. Cowlishaw, D. E. Eyles, W. F. Raymond and J. M. A. Tilley (*J. Sci. Food Agr.* **7,** *768, 775 (1956)*). The fresh herbage was partly disintegrated in a hammer mill, then further macerated and pressed in a screw expeller of special design (J. M. A. Tilley, M. L. Barnes and W. F. Raymond, *Farm Mechaniz.* **6,** *481 (1954)*). After coagulation of the liquor at 80°C, the curd was roller-dried and stored.

The concentrates obtained in this way were then analysed and fed to young chicks (at a level supplying 3 per cent of crude protein) as supplements to a basal ration containing only 8 per cent of protein, but well balanced in vitamin, mineral and crude fibre content. The growth response was then compared with that on a positive control ration supplemented with casein, so that their "gross protein value" could be calculated in the standard manner (V. Heiman, J. S. Carver and J. W. Cook, *Poultry Sci.* **18,** *464 (1939)*).

Three concentrates prepared from alfalfa (*Medicago sativa*) gave protein values ranging from 55 to 65 (relative to casein = 100) and in the presence of added cholesterol the values were raised to levels of 70 to 75. This effect was to be expected from the existence in whole alfalfa of a growth-depressant for chicks reversible by cholesterol (D. W. Peterson, *J. Nutrition* **42,** *597 (1950)*). However, a fourth preparation from alfalfa gave a value of 15 which was increased only to 21 on supplementation with cholesterol. Two samples prepared from rye-grass (*Lolium italicum*) and white clover (*Trifolium repens*) gave values of 51 and 32 respectively, and in neither case was performance improved by the addition of

cholesterol. All these findings are in agreement with the earlier results of K. J. Carpenter, J. Duckworth and G. M. Ellinger (*Proc. European Grassland Conf., Paris, p. 243 (1954)*).

In further trials it was shown that the limiting factor for these materials, under the conditions of the "gross protein value" determination, was the amino acid lysine. In view of the known reduction in the availability of lysine in some processed materials, Tilley *et al.* (*loc. cit*) went on to test a series of materials processed in different ways. It was hoped that a suitable treatment might also, in the case of the alfalfa preparations, remove the growth-depressant fraction, which has been shown to be water-soluble (Peterson, *loc. cit*).

Protein curds were produced from leaf juice, by precipitation with steam, at three different pH values, 4.5, 6.7 and 8.2, but there was no great difference in the nutritive value of the final products. Concentrates prepared from alfalfa were washed with water after precipitation, and this did increase their value for chicks, but not to higher values than were obtained with "unwashed material plus cholesterol". Dried curds were also extracted either with 85 per cent acetone or 90 per cent ethanol but without any significant beneficial effect. However, a sample prepared by freeze-drying did give a value of 59, which was significantly higher than a value of 48 given by control material prepared by ordinary oven-drying.

Other processing variables were also investigated, and leafy crops were processed at different stages of growth, but the authors have been unable to recommend any procedure that will give a consistent, high-quality product. This is a disappointing result in view of the hopes that leaf proteins, being aggregates of tissue rather than storage proteins (as in oil seeds) might prove to be nutritionally superior for building animal tissues (Pirie, *loc. cit.*). M. Davies, W. C. Evans and W. H. Parr (*Biochem. J.* **52,** xxiii) found several leaf-protein preparations to have a low digestibility for rats, and this may also have reduced their value in chick-feeding experiments. Possibly the problem is one of removing waxy complexes that protect organised protein units in the tissues.

Taking short-term considerations, the extraction of protein from leaves has been calculated to be uneconomical. Nevertheless the inefficiency of the series of transfers of leaf protein to seed protein to animal to man remains a challenge to biochemical engineering. A successful outcome conceivably could be of even greater practical importance in some parts of the world than the development of soyabean husbandry and technology has been in the Mid-West of the United States.

AN ANTITHYROID FACTOR IN MILK

It is generally conceded that a dietary deficiency of iodine is the most important etiological factor in the development of simple endemic goiter. This view is supported by the determination of the amount of iodine in the water and foods of endemic areas and the decrease in the incidence of goiter which generally follows the addition of iodine to foodstuffs. The disturbances in iodine metabolism occurring in Mendoza, Argentina, have been investigated using radioactive iodine (*Nutrition Reviews* **10,** *167 (1952)*). An increased thyroid uptake and an apparent prolongation of the biological half-time of thyroid iodine were observed. These observations were interpreted to be the results of simple iodine deprivation.

Despite the established importance of iodine intake in determining the incidence of simple goiter, certain well established dis-

crepancies have been described and have led to the view that substances may occasionally be present in the food or water which block the availability or utilization of dietary iodide. A well-documented example, where such agents have been implicated, has been reported from Tasmania by F. W. Clements and J. W. Wishart (*Metabolism* **5,** *624 (1956)*). In 1949 a survey was conducted of the incidence of simple goiter on the island which showed its general prevalence. The frequency of goiters at different age groups varied; the condition was most common in girls 15 to 17 years of age, of whom 23 per cent were affected. The peak incidence in males was lower (6 per cent), and occurred earlier at ages of 12 to 14 years.

On the basis of this survey the administration of prophylactic potassium iodide tablets to school children was recommended. After this program had been in effect for five years, a subsequent survey was conducted to determine the effectiveness of iodine supplementation on the incidence of simple goiter. Surprisingly, a marked increase in the incidence of simple goiter was found in each age group for both boys and girls, with the exception of girls over the age of 12 years. The incidence of goiter among younger boys and girls was similar, whereas previously there had been a marked preponderance of it among girls. No correlation was found between the incidence of goiter and the enthusiasm with which school officials had cooperated with the supplementation program.

When a detailed analysis was carried out of the change in incidence in goiter in various districts, a highly variable pattern was obtained. In 3 districts a fall in the relative incidence of goiter occurred, indicating that a significant amount of goiter had been due to iodine deficit. The observed increase in the overall incidence of goiter could be attributed to a sharp increase in 2 or possibly 3, areas. To explain this unexpected finding, the possibility was considered that antithyroid substances were present in the foodstuffs of these areas. Although members of the brassicae group of vegetables are eaten in Tasmania, they are all cooked prior to being served. M. A. Greer and E. B. Astwood, (*Endocrinology* **43,** *105 (1948)*) (*Nutrition Reviews* **8,** *196 (1950)*), have shown that the natural antithyroid substance, L-5-vinyl-2-thiooxalzolidone is only released enzymatically when the vegetable is macerated and allowed to stand prior to cooking. Vegetables cooked without maceration do not have an antithyroid action.

Clements and Wishart have given serious consideration to the possibility that antithyroid substances are transmitted from brassica to children by way of cow's milk. The production of milk in this island has increased greatly under government support. During the winter it has been customary to supplement the diet with brassica consisting in the main of turnips, rutabaga and mangels. Recently the marrow-stemmed kale, known as chou-moellier (*Brassica olenacea moellerii*) has been used to a much greater extent for winter fodder for cattle. The greatest increase in the use of this fodder occurred in the areas of the greatest incidence of goiter.

The antithyroid potency of milk from cows fed chou-moellier was demonstrated in human volunteers, rats, and mice by measuring the inhibition of radioactive iodine uptake according to the methods developed by Greer and Astwood. A similar action by the milk of pasture fed cows was not observed. The antithyroid activity was found to be present in alcohol-soluble extract of the milk serum conforming to the solubility of the natural brassica goitrogen.

The thyroid glands of calves whose mothers had been fed chou-moellier showed a decrease in colloid, an increase in follicle cell height, and other changes of hyperplasia.

Clements and Wishart have proceeded to show that, when cou-mollier was mascerated and allowed to stand, an antithyroid substance could be extracted using the method of Greer and Astwood. The chemical

structure of the antithyroid substance from chou-moellier remains to be established. Preliminary studies indicate that milk from cows grazed on pastures heavily contaminated with cruciferous weeds may also contain antithyroid substances, but the activity of this milk is considerably less than that observed in milk from cows fed chou-moellerii.

Clements and Wishart have made a real contribution to our understanding of goiter. The reader cannot fail to be impressed with the skill with which the authors use nutritional, epidemiological, agricultural, radiobiological, and biochemical evidence to strengthen this intriguing hypothesis. Such versatility is rare in the present age of scientific specialization.

The importance of this work in understanding simple goiter is that it bridges the gap between the demonstration of a natural goitrogen in brassica by Greer and Astwood, and the occurrence of human disease. It was always difficult to conceive of any significance of a goitrogen if activity depended upon eating large quantities of raw rutabaga. Although Greer and Astwood were successful in getting volunteers to ingest such foodstuffs, the natural selection of such a diet by human beings seems to be excessively rare. Cows appear to be much less fastidious and the present evidence suggests that they can transmit the goitrogen in their milk. The frequency with which such a mechanism is significant in the development of human goiter remains to be established.

PROTEIN, ASCORBIC ACID DEFICIENCY AND WOUND HEALING

New tissue formation occurring in growth, regeneration, or wound healing requires the presence of adequate amounts of an assortment of vitamins, hormones, amino acids and other organic and inorganic nutrients. The discovery of the presence of a substance in growing tissue is frequently followed by attempts to determine how much of it is required for optimal growth. A similar principle has been applied in an effort to determine the best conditions for the healing of wounds. Criteria which may be used to elucidate the nature and extent of healing include the determination of the tensile strength of the union and the evaluation of the chemical and cellular reaction in the area. J. E. Dunphy, K. N. Udupa, and L. C. Edwards (*Ann. Surg.* **149**, *304 (1956)*) have been studying the nature of wound repair in normal, protein deficient or scorbutic animals using these criteria. They have quantitated the components of the healing wound chronologically.

Determination of the quantity of the tough component, collagen, in the healing wound was made by analysis for hydroxyproline which occurs mainly in collagen. The gel-like embedding material called ground substance was detected by observing metachromasia, the phenomenon in which tissues are stained pink using a basic blue dye such as toluidine blue. This color change is thought to be due to the presence of an acid sulphated polysaccharide which occurs in embryonic connective tissue and in ground substance during healing. Hale's colloidal iron stain was used as a second histological way of observing ground substance mucopolysaccharides. Hexosamine determinations offered chemical means of quantitating ground substance. Silver stains which were specific for reticulin or precollagen material were also used.

The normal sequence of repair described previously (J. E. Dunphy and K. N. Udupa, *New Engl. J. Med.* **253**, *847 (1955)*; *Nutrition Reviews* **14**, *1952 (1956)*) suggests that the early period of healing is one in which ground substance is collecting in large amounts as evidenced by increases in hexosamine concentration in the wound confirmed by the histochemical demonstration of in-

creased metachromasia and colloidal iron staining. Large numbers of macrophages and fibroblasts appear in the granulation tissue along with invading capillaries. Silver staining reticular material is evident during the fifth and sixth days with collagen formation proceeding from the sixth day on. As the proportion of collagen increases, the tensile strength of the wound increases and the hexosamine concentration declines sharply. After 9 days the fibroblasts are mature and collagen fibers are abundant whereas the reticulin fibers have diminished along with the amounts of metachromatic and colloidal iron reacting substances. At 12 days fibroblasts have diminished in number, colloidal iron stains are negative, but the collagen content has increased to a maximum value.

Studies of the localization of S^{35}-labeled methionine in the healing wounds of protein-deficient rats have been reviewed recently. (*Nutrition Reviews* **13,** *218* (*1955*)). The influence of methionine on the above mentioned sequence of the repair process has been studied in rats undergoing a brief protein-depletion both before and after wounding (K. N. Udupa, J. F. Woessner and J. E. Dunphy, *Surg. Gynec. Obstet.* **102,** *639* (*1956*)). Animals on a protein-free diet show a depressed inflammatory response to wounding with fewer macrophages and fibroblasts present after 3 days. The intensities of the staining reactions for ground substance are less than normal and are confirmed by a decreased hexosamine content determined chemically. After 6 days histological change is not striking but there is a continued slow rise in hexosamine content. After 9 days, in contrast to the normal animal, which characteristically shows a diminishing concentration of mucopolysaccharide, the hexosamine content in the wound of the protein-deficient animal is still rising. The 12-day appearance is similar to that of a normal 3 or 6 day wound with no collagen fibers evident. In contrast the addition of methionine to this protein-free regimen leads to a qualitative shift of all these characteristics toward the normal pattern of events. The effect of methionine may be to improve the pattern of amino acids derived from the animal's own muscle or liver protein breakdown so that a more suitable medium for wound healing will be available. In addition cystine, which is stimulatory to chick fibroblast cultivation *in vitro*, arises from methionine *in vivo* and may have a similar role in mammalian fibroblast proliferation.

Guinea pigs were used in experiments studying the effect of vitamin C deficiency on the dynamic process of wound healing (*Ann. Surg.* **149,** *304* (*1956*)). Twenty days of deficiency preceded the preparation of interscapular wounds about 3 cm. in diameter. The animals were killed with ether 4, 8, 12 and 16 days following wounding. Samples were taken carefully to avoid most normal tissue and the chemical and histological examinations were made. Eight of the animals were given 25 mg. of ascorbic acid intramuscularly in the eighth day, and half were killed at 9 and 12 days to observe the effect on the hexosamine and hydroxyproline contents. The normal pattern of healing in the guinea pig was similar to that in the rat and was characterized by an early rise in hexosamine content in the first 4 days at which time collagen was evident chemically as hydroxyproline but not histologically as fibers. Fibroblasts were present as well as positive staining intercellular substance. By the eighth day reticular staining material as well as collagen fibers were present, and the hexosamine concentration had begun to decrease. At the twelfth day the area had become filled with collagen fibers whereas reticular staining and mucopolysaccharide material was no longer positive. The sixteenth-day collagen content differed little from the twelfth-day except that the fibers appeared more dense and mature.

In the scorbutic guinea pigs the fourth-day

tissues appeared to be qualitatively normal except for the absence of metachromatically staining intercellular substance and a decreased amount of hexosamine. On the eighth day the fibroblasts appeared less well oriented, and were large and immature with no evidence of collagen in the area. Reticular material was thick and poorly oriented and hexosamine had increased rather than decreased. By the twelfth day fibroblasts were still immature, ground substance was stained, reticular material was abundant, hexosamine content remained high, and collagen fibers were absent. The findings on the sixteenth day differed little from the previous samples and the animals had become ill so that further sampling was discontinued.

In contrast, the animals given ascorbic acid showed marked alterations in hexosamine and hydroxyproline contents indicating that the vitamin deficiency prevented the accumulation of hexosamine and the synthesis of collagen.

This study indicates that the difference between normal repair and repair in protein-deficiency is apparently due to a lack of essential precursor materials in the latter and may be corrected by the simple addition of methionine. In contrast, during vitamin C deficiency the precursor materials are present but "a key to collagen synthesis is lacking". Both deficiency states and the work in both the guinea pig and the rat indicate the importance of ground substance changes preceding fibroblast proliferation.

FUNCTION OF VITAMIN E IN REPRODUCTION

The work of H. Dam and his collaborators has demonstrated that the absence of vitamin E from the diets of rats and chicks produces certain symptoms which appear to be direct results of the oxidation of the depot and liver fat of these animals. Thus, with a diet deficient in vitamin E but containing some highly unsaturated fat, such as cod liver oil, both these species consistently develop pigmentation and peroxidation of the body fat and destruction of liver vitamin A. One question which arises from a consideration of these observations is whether other well known symptoms of vitamin E deficiency, such as sterility, liver necrosis, and lung hemorrhage are also the results of the absence of the apparently nonspecific antioxidant action of this vitamin, or whether some more specific function may be assigned to it.

Some of the more recent results of Dam and his co-workers seem to indicate the former conclusion, since several apparently unrelated compounds, such as methylene blue, ascorbic acid and antabuse (tetraethylthiuram disulfide), prevented rats from developing such symptoms of vitamin E deficiency as fat coloration and peroxidation, incisor depigmentation, hepatic damage and sterility (H. Dam, H. Granados, and I. Prange, *Acta physiol. Scandinav.* **18,** *161 (1949)*; *Nutrition Reviews* **10,** *149 (1952)*). In spite of these discoveries, the original question remained unanswered since it was not apparent from these studies whether these active compounds replaced alpha-tocopherol directly, or whether they had some indirect action. Neither was the mechanism of action of vitamin E in preventing some of the symptoms any more apparent. However, two observations pointed the way to further experiments. First, the methylene blue and antabuse were effective only if cod liver oil were the fat used to develop the symptoms, thus indicating that at least part of their effect may lie in detoxification of some substance in this oil. Second, growth depression occurred with both compounds.

The results of some of these experiments have been reported recently. F. Christensen and H. Dam (*Acta physiol. Scandinav.* **36,** *82 (1956)*) have shown that on a strictly

fat-free diet (vitamins A and E given as an aqueous emulsion instead of in peanut oil which furnished 3 mg. of linoleic acid per day per rat) vitamin E and the other compounds (antabuse, methylene blue and nordihydroguaiaretic acid) were much less effective in preventing sterility in the rats than they were when the peanut oil vehicle was used. Moreover, the latter two synthetic compounds again showed some toxic effects. When they were fed as 0.126 and 0.5 per cent of the diet, respectively, growth of the rats decreased and early death resulted in many cases.

In previous studies (H. Dam and H. Granados, *Acta pharmacol. et Toxicol.* **8,** *47 (1952)*), it had been found that with diets containing 20 per cent lard, and possibly on fat-free diets, methylene blue had a beneficial effect on reproduction even in the absence of vitamin E. In order to ascertain whether the effect of lard was due to the fat itself or to its content of vitamin E, a series of diets was formulated using lard from which the vitamin E (about 8 micrograms per gram) had been removed by chromatographic adsorption on filtrol (F. Christensen, H. Dam, and R. A. Gortner, *Acta physiol. Scandinav.* **36,** *87, (1956)*).

Five diets contained, respectively, no fat, 20 per cent "crude" lard, 20 per cent chromatographed lard, and 2 per cent of each type of lard. Each of these diets was subdivided into three groups containing no supplement, 0.02 per cent DL-alpha-tocopherol acetate, or 0.126 per cent methylene blue, respectively. After 62 days on the fat-free, tocopherol-free diet, female rats were divided into 15 groups of 8 each, corresponding to the experimental diets. After 21 days on the experimental diets, they were mated with normal males. The rats which were continued on the fat-free diets developed obvious symptoms of essential fatty acid deficiency. Only 3 young were produced and only 14 of the 24 rats survived the experiment. These groups furnished little information beyond confirmation of the well known effects of fat deficiency.

When 20 per cent untreated lard was added to the diet, symptoms of essential fatty acid deficiency disappeared and reproduction was normal except in the unsupplemented diet in which no young were born. With 20 per cent chromatographed lard, the results were the same except that the methylene blue-supplemented group produced only 6 young (as compared with 43 young for those on the untreated lard). The two groups on the 2 per cent lard diets were similar in that essential fatty acid deficiency symptoms were not relieved and reproduction was normal only with vitamin E supplementation.

The most significant observation from these experiments is that methylene blue had a favorable effect only in the presence of small amounts of vitamin E (20 per cent untreated lard), thus suggesting that methylene blue may owe its activity to a protective action on vitamin E rather than to a generalized antioxidant effect.

In an effort to determine the substance protected by vitamin E, the authors tested the effect of increased casein or vitamin B_{12} in the vitamin E-deficient diet (F. Christensen and H. Dam, *Acta physiol. Scandinav.* **36,** *97 (1956)*). Since no effect was observed, it seems probable that the effect of vitamin E is not in the protection of these substances.

Thus it can be seen that, although much of the action of vitamin E may be ascribed to its property as an antioxidant for lipids in various body tissues, certain of its functions are probably more specific. Since other antioxidants cannot replace vitamin E in these functions, it is probable either that some structural specificity or solubility properties are necessary for a specific antioxidant action or that vitamin E may be involved in some functions which are not primarily antioxidant. Further experiments should involve the locus of the action of this vitamin in some of these functions, such as reproduction capacity.

It seems very likely that the fundamental role of vitamin E (d-alpha-tocopherol) has been clarified substantially by the recent report of A. Nason and I. R. Lehman (*J. Biol. Chem.* **222,** *511* (*1956*)) in which vitamin E is cited as a co-factor in the electronic transfer between cytochrome b and cytochrome c.

GALACTOSE UTILIZATION IN SUCKLINGS

Rats given experimental diets containing large amounts of lactose or galactose excrete considerable quantities of the latter in the urine. Growth on such diets is slight, the animal presents a disorderly appearance, and often the lenses of the eyes assume a typical cloudiness (B. C. Guha, *Biochem. J.* **25,** *1385* (*1931*); P. H. Derse *et al.*, *Proc. Soc. Exp. Biol. Med.* **66,** *545* (*1947*); P. Fournier *et al.*, *Soc. Biol. Paris* **148,** *265* (*1954*); *Nutrition Reviews* **4,** *204* (*1946*); **6,** *185* (*1948*)). Biochemical changes include a decrease in body fat and increases in water content and total ash. Increasing the proportion of fat in the diet may result in a decreased concentration of sugar in the urine and less cloudiness of the lenses (L. K. Riggs, *Arch. Ophth.* **51,** *415* (*1954*)).

In animals which do not suckle it has been shown that lactose is completely split in the digestive tract and, indeed, that the galactose moiety of the molecule is more rapidly absorbed than is the glucose. Some galactose is actually converted to glucose (R. Caputto *et al.*, *J. Biol. Chem.* **184,** *333* (*1950*)). However, such conversion appears to be limited in rats, at least in adults and weanlings, which may explain the low galactose tolerance of this species.

Since lactose is the only carbohydrate available to sucklings, it is possible that the metabolism of galactose differs in suckling, weanling and mature rats, and that tolerance is high in sucklings. To investigate such possibilities, A. Scheunert and H. Sommer (*Biochem. Z.* **327,** *461* (*1956*)) have studied the effects of various carbohydrates, especially galactose, upon growth and body constituents during fairly early stages of postnatal development.

In an initial experiment groups of young rats weighing between 40 and 50 g. were fed diets containing respectively 65 per cent each of the following carbohydrates: dextrin-maltose, dextrinized starch, sucrose, lactose, or galactose. It was found that very slight growth occurred in groups fed galactose, and 3 of the 5 rats in these groups died; 7 of 10 rats on the lactose diet also died during the experiment. Water content of the carcasses was slightly elevated in the lactose- and galactose-fed groups as compared to the group fed sucrose. Fat content was lower in the former groups and the total body ash contained higher concentrations of calcium and phosphorus but in the normal ratio. The dextrin-maltose diet resulted in an especially rapid growth (addition of 92.2 g. total weight as compared to 74.7 g. in sucrose-fed and 12.0 g. in galactose-fed animals) and relative decreases in total body water, total ash, phosphorus and calcium.

When rats weighing 45 to 55 g. were given 6 and 12 per cent solutions of lactose in milk, 15.7 and 18.7 per cent of the total ingested galactose appeared in the urine as compared to 4.6 and 3.1 per cent excretion in animals fed equivalent percentages of glucose in milk.

More critical evaluation of galactose tolerance revealed that suckling rats can utilize galactose completely when it is present in no larger proportion than 5 per cent of the total diet by dry weight. If fat or protein rather than glucose is present as a high per cent of the diet, the tolerance can be elevated to approximately 10 per cent, the criterion for tolerance being the appearance of galactose in the urine. Young rats fed an artificial

mixture of 44 per cent fat, 39 per cent casein, 5 per cent salt mixture, and 12 per cent lactose nevertheless contained 10 per cent less total liver lipid than did rats fed a similar diet except for the substitution of glucose for lactose. Unfortunately no data from the above experiments relate the absolute rather than relative amounts of galactose in the diet to the changes reported.

When comparison is made of the lactose content of rat's milk with that of other animals, it is found that the former contains only about 9 per cent lactose, whereas cow's milk contains about 38 per cent lactose and human milk contains almost 57 per cent. This may indicate that galactose tolerance differs widely among mammalian sucklings. Apparently, suckling as well as adult rats differ from human and bovine species in that they lack the capacity to utilize galactose as a major source of energy (galactosemia occurs only very rarely in human infants). Indeed, it was reported by Scheunert and Sommer that when galactose is withheld from 40 g. suckling rats, development takes place readily in the presence of glucose. Thus the role of this sugar in development remains obscure. Perhaps other species are more dependent on its presence in the diet than is the rat. It has been reported that milk sugars aid in the absorption of calcium, a finding which is supported by the present studies on mineral deposition but seems to be contradicted by the X-ray studied of P. Handler (*J. Nutrition* **33,** *221* (*1947*)). It is further suggested that galactose is involved in the development of the central nervous system, in which there is intensive activity in many species during the first few postpartum days. It should prove illuminating to study the effects of galactose deprivation in very early sucklings, a study which has not been carried out in the rat because of the difficulty of handling such small animals. Moreover, since the rat may present a peculiar case with respect to utilization of galactose, such studies might better be performed on newborn calves or on other mammals with high galactose tolerance.

FAT SOLUBLE VITAMINS AND ATHEROSCLEROSIS

The possibility that vitamin E might be active in preventing the formation of atheromatous plaques has tempted many investigators to conduct experiments in this field. These investigations have seemed justified particularly in view of the report of Glavind and co-workers (*Acta Pathol. Microbiol. Scand.* **30,** *1* (*1952*)) that the peroxide numbers of the arterial lipid deposits are extraordinarily high, reaching 30 (milliequivalents per kg.) in advanced cases. The use of this vitamin in clinical studies has, however, invariably resulted in inconclusive or negative results (*Nutrition Reviews* **5,** *164* (*1947*); **7,** *118* (*1949*); **7,** *314* (*1949*)).

Animal experiments have also generally indicated vitamin E to be ineffective in retarding the development of arteriosclerosis. H. Dam (*J. Nutrition* **27,** *193*, *289* (*1944*)) and J. Stamler, R. Pick, and L. Katz (*Circulation* **8,** *455* (*1954*)) observed no definite effect of this vitamin, whereas M. Bruger (*Proc. Soc. Exp. Biol. Med.* **59,** *56* (*1945*)) and J. P. Levinson (*Angiology* **3,** *397* (*1952*)) have actually reported definite increases in sclerotic lesions when vitamin E was administered to cholesterol-fed rabbits. On the other hand M. Baguena *et al.* (*Arch. Med. Exp.* **14,** *47* (*1952*)) have claimed antisclerotic effects with alpha tocopherol.

Few studies have been concerned with the role of vitamin A in atherosclerosis. In one reported experiment E. Oppenheim and M. Bruger (*Arch. Path.* **53,** *520* (*1952*)) failed to show significant results in cholesterol-fed rabbits.

During the last few years an extensive

series of experiments has been carried out by G. Weitzel, H. Schön, F. Gerz, and E. Buddecke (*Z. f. physiol. Chem.* **304,** *247 (1956)*) on the influence of vitamins A, E and K on naturally occurring atherosclerosis in old hens. In earlier studies one of these workers (Weitzel) investigated the surface activity of phytol groups and of the vitamins (A, E and K) which contain such branches (Weitzel, A.-M. Fretzdorff, and S. Heller, *Ibid.* **290,** *32 (1952)*; **303,** *14 (1956)*). It was found that vitamin A and its physiologically active derivatives and vitamin E form stable monomolecular films on hydrophylic surfaces, whereas vitamin K is much less active in this respect. A mixture of vitamins A and E also forms monomolecular films spontaneously.

These observations have resulted in new concepts of the manner in which these vitamins may function in biologic systems. It is presumed that in living cells vitamins A and E form monomolecular films on hydrated interfaces, such as are formed by proteins. The intima of blood vessels are such hydrated interfaces and since they are in close contact with vitamins A and E which are transported on the same lipoprotein molecules, it is conceivable that the surfaces of the intima are more or less layered with these vitamins. Exchange properties of the vessels may be related to their vitamin content. As a test of these notions, the authors attempted to determine if vitamins A, E and K influenced the lipid exchange properties of the intima, and specifically whether atheosclerotic lesions might be affected.

In the studies of Weitzel and co-workers, old atherosclerotic hens and a few groups of young cholesterol-fed cockerels were given large oral doses of vitamin K_1, vitamin E (DL-alpha-tocopherol-acetate) and vitamin A palmitate for periods ranging from 75 to 100 days. The individual aortas were assessed macroscopically, and analyzed for their lipid content; the lipids of the liver and serum were also isolated. Free and esterified cholesterol and lipid phosphorus were determined separately on all total lipid fractions.

Thirty mg. per day of vitamin K for 109 days proved ineffective in reducing atheromata in old hens. Lipid extracts from the livers of vitamin K-treated birds showed a slight elevation in concentration, but the ratios of free to esterified cholesterol were the same as in control birds.

When vitamin E was administered alone in daily oral doses of 50 mg. for 75 days, slight reduction of atheromatous deposits was noted. A lipotropic effect on the liver, similar to that of phytol, was anticipated but was not observed. There was no detectable change in the cholesterol content of the liver as compared to controls, but analyses of serum revealed increases in both free and esterified cholesterol.

Significant decreases in lipid deposits of the aorta were observed upon oral administration of 10 mg. of vitamin A per day. The total lipid of the aortas was reduced, but it was noted that the decrease was almost exclusively in the neutral fat component. Total cholesterol in the aortas remained at the same levels as in untreated controls. The influence of vitamin A on the liver was a shift in the free to esterified cholesterol ratio from a normal value of 5:1 to 1:1, but total cholesterol was present in concentrations similar to those in control animals. No lipid changes were observed in the serum with this treatment.

Combined dosage of vitamins A and E (7 mg. of vitamin A and 35 mg. of vitamin E or 10 mg. of vitamin A and 50 mg. of vitamin E per day) was found to lessen the prevalence of fatty plaques in old hens. This was accompanied by decreases of all lipids of the aorta, including cholesterol. The animals in the groups given vitamins A and E, as in certain instances where vitamin A alone was given, displayed definite shifts in the ratio of free to esterified cholesterol in the liver. In all instances esterified cholesterol

was elevated. Lipid analyses of the serum revealed no characteristic differences as compared to untreated control hens.

Atheromatous lesions are known to contain relatively high concentrations of organic peroxides (J. Glavind *et al., loc. cit.*). According to Weitzel and co-workers, this situation indicates normally low rates of exchange in the atheromata and further suggests a possible role for vitamin E. In the absence of vitamin E, the vitamin A coming in contact with lipid peroxides may be largely oxidized. However, if vitamin E were present it could act possibly to reduce the oxidized vitamin A or, more likely, prevent its oxidation.

The above results indicate the absence of quantitative correspondence between lipids present in the aorta and those circulating in the plasma, since vitamin-treated and control birds had similar amounts and proportions of serum lipids. This does not, however, rule out the possibility that the rate of removal may be a function of the concentrations of vitamins A and E. The preferential mobilization of neutral fats from the aorta by administration of vitamin A alone, as observed in these experiments, would be a significant observation if confirmed and established as a general phenomenon.

In assessing the significance of these results it is well to recall two facts. First, these are the first experiments of several attempts which suggest that vitamins A or E may play a role in the lipid metabolism of atheromatous plaques. Second, it must be remembered that the response of the hen to these vitamins may be quite different from that of the human being or even other experimental animals. However, the advancement of new ideas relative to the basic etiology of this widespread disease, should cause many investigators to conduct experiments which will confirm or deny these suggestions.

PROTEIN STRUCTURE AND PROTEIN SYNTHESIS

One of the most difficult problems of modern science is to gain an understanding of how the complex protein molecule is fabricated by living cells. Nutritionists, physicians, biochemists and many other groups will be able to utilize additional knowledge of this process to study important problems in health and disease. It is particularly helpful to find the present status of knowledge in this field summarized at frequent intervals.

The "Symposium on the Structure of Enzymes and Proteins" (*J. Cell. Comp. Physiol.* **47,** *Suppl. 1 (1956)*) is a collection of current reports of specialists endeavoring to work out the structures of proteins and the mechanisms concerned with their synthesis.

The report by H. Borsook on the biosynthesis of peptides and proteins summarizes our present information about the mechanism of synthesis and points out the need for a clearer picture at the molecular level. According to Borsook three different phases may be involved in protein synthesis: (1) the activation of the carboxyl group of the amino acids, (2) the transport of activated amino acids to the nucleic acid template where arrangement in specific sequence occurs, and (3) the linkage by peptide bonds and the peeling off from the template. Shortly after the third step the specific coiling must occur, governed either by the amino acids, or an inducer in the case of adaptive enzyme formation, or by an antigen in the case of antibody formation. The activation of carboxyl groups in the synthesis of quasipeptides, amides, or small peptides may conceivably apply to the biosynthesis of proteins. As an illustration,

the reaction in which benzoic acid combines with glycine to form hippuric acid has been studied. Here it is seen that the free energy gain is of the same order of magnitude typical of peptide bond synthesis and requires a free energy donor. The postulate includes the utilization of energy from ATP (adenosine triphosphate) in the formation of an enzyme-ATP complex which unites with coenzyme A which in turn forms a benzoyl-coenzyme A complex capable of coupling with glycine.

Activation and coupling occur in the synthesis of other peptides such as pantothenic acid, glutamine and glutathionine. The theory of analogous carboxyl activation as a part of the mechanism of protein synthesis has received support in several types of experiments. One type of evidence for this activation mechanism is the *in vitro* acceleration of incorporation of amino acids into liver microsomal proteins in the presence of supernatant enzymes and ATP. A second type of evidence comes from the evaluation of the requirements as well as the effect of inhibitors on adaptive enzyme synthesis. Synthesis requires an energy source in addition to the amino acids and the inducer, whereas inhibition of oxidative phosphorylation also inhibits adaptive enzyme formation.

The well known term "specific dynamic action" refers to the large increase in basal heat production (about 30 per cent) which is a manifestation of energy lost as heat following the metabolism of a protein meal. This phenomenon suggests the possibility that large amounts of energy are required for the metabolic transformations of amino acids and that some of this is wasted, perhaps because the rate of ingestion is too rapid or the amino acid pattern is incomplete.

Isotope studies using labeled amino acids should assist in the assessment of the nature of the intermediates involved in protein synthesis. If a labeled amino acid present in several different loci in a protein still shows the same isotope abundance, the conclusion would be feasible that all amino acids of the protein are in equilibrium with the amino acids of the medium. If peptides, either free or bound, are intermediates or if reversible reactions take place, an unequal labeling of the protein might result, as postulated by C. B. Anfinsen and D. Steinberg (*Fed. Proc.* **10,** *156 (1951)*). Failure to find peptide intermediates as well as the inability of other workers to produce unequal labeling make it difficult to conceive of peptide intermediates in this phenomenon. The results of experiments in antibody formation and adaptive enzyme synthesis support this view.

The author proposes a scheme of protein synthesis in which activated amino acids unite with the phosphate radical of nucleic acid by their carboxyl groups under the influence of one enzyme, followed by peptide bond formation and removal from nucleic acids under the influence of a second enzyme.

The fact that cell fractions with the highest RNA (ribose nucleic acid) concentrations have the fastest rate of incorporation of amino acids into protein and that protein synthesis in cell-free preparations of *Staphylococcus aureus* is abolished by ribonuclease tends to implicate RNA in protein synthesis. Study of the relation between RNA synthesis and protein synthesis has revealed that the presence of RNA does not guarantee protein synthesis, that when protein synthesis is going on RNA turnover also occurs and when RNA synthesis is inhibited protein synthesis stops.

How the shallow RNA helix can bind the steep helix of protein without strain has caused some speculation. One solution to the difficulty would be the binding of only carboxyl groups. Another possibility would be that the bases which bind the amino acids would be widely separated by as much as 10 nucleotide units.

The rate of protein synthesis depends on the rates of the various reactions which are

involved; for example, the rate of activation or the rate of ATP synthesis, as well as on the concentrations of the free amino acids. Protein synthesis fails in the whole animal when a single essential amino acid is lacking, as in the situation where a poor quality protein is fed. The extent of synthesis depends on the fraction of the requirement of any essential amino acid which is present in limited amount. In the animal this quantity is the sum of the requirements for synthesis of many different proteins with differing amino acid patterns. The eating of a poor quality protein for breakfast followed by eating a protein of different poor quality for lunch may not serve to fill the complete amino acid need for adequate protein synthesis. Apparently there is no pool of amino acids large enough to survive the continuous catabolic process while waiting for a delayed supplement of the missing amino acids.

Protein synthesis has been inhibited by amino acid analogues as evidenced by growth studies in microorganisms or experimental animals. One highly provocative outcome of the derangement of protein synthesis by incomplete amino acid mixtures or antibiotics is the accumulation of peptides, which occurs perhaps only in microorganisms where protein synthesis is very rapid.

The exchange of amino acids in proteins could mean a process of unequal labeling by a given isotopic amino acid at different loci in the protein molecule as a result of the slow synthesis of protein. A second kind of exchange in which an amino acid may be incorporated into protein is one in which "synthesis of protein *de novo* from amino acids is excluded." This type of exchange could be explained if it were assumed that the preformed protein could be reimposed on the template, at least in part, with exchange occurring only with those amino acids attached to the template.

The term "protein turnover" originally applied to the exchange of labeled for unlabeled amino acids in the proteins of animals. The finding that the proteins of an animal exchange amino acids with those in the metabolic pool even though the animal is in nitrogen balance has led to the conclusion that proteins are being continually broken down and rebuilt. The discovery that induced beta-galactosidase of *E. coli* is very stable, however, led D. S. Hogness *et al.* (*Biochim. et biophys. acta* **16,** *99* (*1955*)) to question the concept of the dynamic state of proteins (*see Nutrition Reviews* **13,** *237* (*1955*); **14,** *53* (*1956*)). Now the realization that the rate of synthesis and breakdown of protein varies in different cell fractions and in different proteins necessitates a redefinition of the term "rate of protein turnover" in terms of individual proteins rather than for all the proteins of an organism.

The value of this symposium for the biologist is to help him realize that the physical chemists and biochemists are capable of great accomplishment in explaining protein synthesis at a molecular level. Soon the nebulous descriptive terms may be replaced with a clear picture of a reaction mechanism. This will undoubtedly help the biologist in the solution of many problems.

WATER EXCHANGE IN RATS

In spite of the interest in water exchange, both from the point of view of the central control of water intake and balance and from the point of view of abnormalities of water exchange such as famine edema, remarkably few data are available on the water intake and balance of laboratory animals.

A general picture is shown by Strominger (*Yale J. Biol. Med.* **19,** *279* (*1946/47*)) who confirmed that the water intake of rats bears

a close relationship to their food intake. In his experiments, water intake was found to be approximately twice the food intake by weight (or ⅔ of the caloric intake) and this relationship was maintained even when the rats were made experimentally hyperphagic. H. M. Bruce and G. C. Kennedy (*Proc. Roy. Soc. s. B* **138,** *528 (1951)*) found a tendency to a constant ratio, but the absolute values of the ratio differed from those found by Strominger. Bruce and Kennedy also found that the absolute intake of water and the ratio altered with the type of diet, the water being higher on a diet high in nitrogen. They did not attempt to establish any numerical relation between water intake and nitrogen, but concluded that the water intake depended on the need of water for renal clearance. E. W. Crampton and L. E. Lloyd (*Nutrition Reviews* **13,** *157 (1955)*) also showed this interdependence of food and water intake in growing rats.

Even if this food-water relationship continues over physiological ranges of intake, it is likely that there is a point where the constant ratio relation breaks down (at some point as the food intake is reduced to zero). The extreme case of the effect of prolonged total inanition on the water intake of rats has been examined by J. Le Magnen (*J. de Physiol., Paris,* **48,** *608 (1956)*). Six groups of 5 rats each were, after equilibration ad libitum, deprived of all food. Two groups were allowed water ad libitum. Two groups were allowed water ad libitum and, in addition, were given water by stomach tube every other day. One group was allowed neither food nor water, and one group was allowed isotonic saline ad libitum.

In all groups the mean water intake was claimed to fall by 70 to 80 per cent on the first day of inanition. With irregularities this residual water intake was maintained for about three days, but then continuously declined until the death of the animals.

K. Huang (*Am. J. Physiol.* **181,** *609 (1955)*) showed that the water intake of rabbits which were deprived of all food can vary from great increases over normal to great decreases. Only half of these animals showed changes in water exchange similar to the changes found in pure salt depletion. Unfortunately, Le Magnen does not give the water intakes for the fed equilibration period. Neither does he give individual values, nor measures of dispersion, for his experimental data. The work does not, therefore, add greatly to the sparse body of available data on the subject. Nevertheless, two salient features make the work of particular interest: (1) The terminal body weight (43 to 45 per cent of initial weight) and the age at death (about ten days) were the same for each group. The total deprivation of water, as well as of food, had no deleterious effect on survival. Although the saline-fed group maintained their residual water intake for about seven days of starvation, the time and weight at death did not differ from those of the other groups; (2) The residual water intake was not constant but, after initial irregularities, declined continuously to the time of death.

From the first of these findings, Le Magnen suggests that the residual water intake during fasting is useless since neither its augmentation nor complete removal alters the terminal picture. Bruce and Kennedy's contention that the water intake is dependent on renal needs, is held by Le Magnen to be a possible explanation of his findings. Certainly such a physiological explanation is more attractive than the purely empirical relation shown by Strominger. It is difficult to see, however, how this can explain the failure of water deprivation to have any effect on the survival of starved rats unless the nitrogen metabolism or, less likely, the extra-renal routes of water loss differs markedly from that in pure inanition, or unless both the normal intake and the residual intake of inanition contain a large training or conditioned moiety which is not strictly necessary.

METABOLIC ALTERATIONS AND EXPERIMENTAL FUNGUS INFECTIONS

Mucormycosis is a fungus infection which is usually found in association with metabolic disorders, particularly diabetes mellitus. Bauer and associates (*Am. J. Med.* **18,** *822 (1955)*) and J. S. Harris (*Pediatrics* **16,** *857 (1955)*) have reported the isolation of the phycomycetes Rhizopus oryzae and Rhizopus arrhius from two diabetic patients with cerebral mucormycosis. H. Bauer, J. F. Flanagan and W. H. Sheldon (*Yale J. Biol. Med.* **28,** *29 (1955)*), and D. T. Elder and R. D. Baker (*Arch. Path.* **61,** *159 (1956)*) have reported that with these species of Mucorales it is possible to produce cerebral and pulmonary lesions in rabbits made diabetic with alloxan. The experimental lesions resemble the cerebral and pulmonary lesions found in man with mucormycosis. Nondiabetic rabbits show only rare, minute lesions at the site of inoculation in the nostrils. Thus, it appears that these normally saprophytic organisms may become pathogenic when present in an animal having severe metabolic alterations.

Recently H. Bauer, J. F. Flanagan and W. H. Sheldon (*Yale J. Biol. Med.* **29,** *23 (1956)*) studied the effects of inoculating rabbits with this type of fungus at various times preceding the production of alloxan diabetes to determine if the change of this organism to a pathogen is dependent upon the presence of metabolic alterations at the time of infection. To this end, 19 male rabbits were inoculated in one nostril with a standard suspension of Rhizopus oryzae spores. Acute diabetes was produced in five of these rabbits by giving alloxan 24 hours after the introduction of fungus spores. Nine rabbits received the alloxan 96 hours after being infected with the fungus. Five rabbits, which were infected and did not receive alloxan, served as control animals. The rabbits which received the alloxan twenty-four hours after inoculation with the fungus survived from 60 to 124 hours. These animals all had blood sugar levels between 512 and 924 mg. per cent, and 4 of them had acetonuria. All 5 of the rabbits had nasal lesions and 4 of them presented pulmonary lesions. Definite signs of meningencephalitis were found in 3 of the rabbits which were infected twenty-four hours before receiving the alloxan, and the other 2 rabbits evidenced slight meningeal inflammation.

The animals which were injected with alloxan three days after being inoculated with the spore suspension had blood sugar levels similar to the other diabetic rabbits. Eight of the 9 rabbits had acetone in the urine while the other rabbit showed only traces. These animals survived sixty to one hundred-twenty hours after being given the alloxan. Of the 9 animals treated, nasal lesions were found in 5 and pulmonary lesions were found in 4. None of the animals in this group had cerebral lesions. The control animals which were infected with the fungus, but not rendered diabetic, and sacrificed at intervals to correspond with the death of the test groups, showed only a few lesions of minute size. These lesions consisted of well defined foci of superficial ulceration of the nasal mucosa with many normal-appearing polymorphonuclear leukocytes around the rare degenerating spores. In contrast the rabbits which received the alloxan after being exposed to the fungus had extensive lesions, which were characterized by marked mycelial proliferation and invasion of the tissue, particularly that of blood vessels. Another striking finding in this group of animals was the degenerative changes in the polymorphonuclear leukocytes of the inflammatory reaction.

Since hyperglycemia is one of the characteristic findings in uncontrolled diabetes, a study was undertaken to determine the pathogenicity of this fungus in animals rendered hyperglycemic by the constant infusion of glucose. These animals were given a 10 per cent glucose solution in 0.45 per cent saline which also contained 20

mEq of potassium per liter. The rabbits were immobilized by means of a neck yoke and received the sugar in a marginal ear vein. The rate of infusion was such as to maintain the blood sugar above 400 mg. per cent This level of blood sugar was maintained from forty-five to fifty hours. The animals were inoculated in one nostril with a standard suspension of Rhizopus oryzae spores after the blood sugar was maintained at a level of 400 mg. per cent or greater. As control animals for this series, 4 rabbits were treated in a similar manner but given saline intranasally rather than given spores.

The animals which received the glucose solutions containing the saline showed a slight drop in plasma sodium in the first twenty-four hours which returned to normal within forty-eight hours. The plasma potassium level did not change during the study and the chloride level of the plasma increased about 10 to 15 mEq per liter (normal values were 95 to 105 mEq per liter) after twenty-four and forty-eight hours of intravenous infusions. The carbon dioxide-combining power had fallen to about ½ of its normal value (40 to 50 volume per cent). When infusing a 0.45 per cent solution of sodium chloride, equivalent amounts of sodium and chloride are being added to the blood stream, but since there is about ⅓ more sodium than chloride in the plasma (expressed as mEq per liter) the level of chloride is being increased out of proportion to that of sodium. Thus, unless the animal excretes chloride in excess of sodium, acidosis results.

Nasal lesions were present in 11 of the 12 hyperglycemic rabbits but only 3 showed pulmonary lesions. Six of the animals with nasal lesions had only minute areas showing superficial mucosal ulceration. No cerebral involvements occurred in any of the animals. This series of studies shows that the hyperglycemia and acidosis produced by glucose-saline infusions, and not by primary injury to the pancreas, causes changes in the rabbits which renders the fungus pathogenic. The lesions in the hyperglycemic rabbits differ in both number and size from both the acute diabetic and metabolically normal rabbits, these lesions being less marked than in the diabetic rabbits and more marked than in the control rabbits.

This study stresses again the importance of metabolic aberrations (diabetes and its sequellae) in the pathogenicity of Rhizopus oryzae infections. In both the acute state of alloxan diabetes and the hyperglycemia with decreased carbon dioxide-combining power, there was mycelial proliferation in the tissues of rabbits and degenerative changes in the nucleus of the leukocytes. In the case of acute alloxan diabetes where there was ketonuria, hypernatremia, hyperlipemia, and dehydration, there were massive fungus lesions and invasion of the organism into the blood vessels and infarction of tissues. The possible common basis responsible for lesions in both groups of rabbits with induced metabolic aberrations appears to be an altered leukocyte function, although this does not completely explain the distinctive differences in lesions which is a reflection of the pathogenicity of the organism.

NOTES

Effects of Acetazolamide on Hepatic Bile

Long-continued observation of a patient with an external biliary fistula permitted S. Fink (*New Engl. J. Med.* **254,** *258 (1956)*) to examine the effects of acetazolamide (2 - acetylamino - 1,3,4 - thiadiazole-5-sulfonamide), a carbonic anhydrase inhibitor, on various constituents of hepatic bile. A cholecystectomy had been performed on this patient, and two months after the completion of this study he developed increasing hepatic insufficiency due to biliary cirrhosis. For this reason, the composition of the bile may not be the same as that from a normal liver, although the changes produced by acetazolamide are probably representative. Bile for this study was collected throughout twenty-four hours and a total of 18 collections was averaged for the control values.

The osmotic pressure of the hepatic bile averaged 299 milliosmols per liter, a value slightly higher than that of serum. Bile sodium was likewise slightly higher than serum sodium. The pH value of the bile averaged 7.46. Total lipids, phospholipids and cholesterol were significantly lower than corresponding serum levels. The concentration of the bile cholesterol was 6 to 16 mg. per cent at a time when the serum cholesterol was 142 mg. per cent.

The effects of acetazolamide were investigated in two twenty-four hour trials. It was found not to influence the volume of fistula bile. The effects on the kidney were those usually noted, an increased excretion of bicarbonate, chloride retention and consequent production of an alkaline urine. The serum electrolytes reflected these changes in the production of a hyperchloremic acidosis.

Although liver cells contain carbonic anhydrase, the electrolyte concentrations were not influenced directly by the acetazolamide. The electrolyte pattern of the bile followed that of the serum with a decreased pH and bicarbonate concentration. Thus it may be inferred that carbonic anhydrase is of no significance in the elaboration of hepatic bile.

Insulin Hypoglycemia in Angina Pectoris

The clinical dictum that hypoglycemic episodes constitute a serious hazard to the patient with angina pectoris has been supported by relatively few case reports. W. E. Judson and W. Hollander (*Am. Heart J.* **52,** *198 (1956)*) have made a systematic attempt to document this impression by studying the reactions of patients with arteriosclerotic heart disease and the anginal syndrome to hypoglycemia induced by insulin.

The 11 patients had typical anginal pain on effort, associated with electrocardiographic evidence of myocardial ischemia. Insulin was injected intravenously, 0.1 unit per kilogram of body weight, and the blood glucose, sodium and potassium were measured at fifteen- to thirty-minute intervals. An electrocardiogram and ballistocardiogram were simultaneously recorded. Some of the patients received hexamethonium in a dose sufficient to produce postural hypotension before the insulin was given.

When intravenous insulin alone was used, the blood sugar fell from an average level of 81 mg. per cent to 38 mg. per cent. The serum sodium did not change but the serum potassium decreased an average of 0.7 mEq per liter. Seven of the 11 patients showed electrocardiographic changes which were unlike the myocardial ischemic changes produced by exercise. The most common change was a flattening and broadening of the T waves. In none of these patients, however, was anginal pain produced, although they displayed the typical signs and symptoms of hypoglycemia during the test.

When the patients were pretreated with hexamethonium in an effort to prevent the effects due to adrenergic stimulation, the

electrocardiographic changes and symptoms were not altered.

The effect of this investigation is thus to show that this degree of hypoglycemia does not cause the electrocardiographic changes of myocardial ischemia in patients with arteriosclerotic heart disease. The electrocardiographic changes were consistent with those attributed to hypokalemia (low blood potassium levels) and the decrease in serum potassium supports this idea. No convincing evidence was obtained to suggest that these changes were due to discharge of the sympatho-adrenal system. Although this study allays some of the fears which have previously been emphasized regarding the close control of diabetic subjects with arteriosclerotic heart disease, it should not be assumed that hypoglycemic shock may not, on occasion, cause adverse cardiovascular effects.

Glycogen Metabolism

It is known that the molecular weight of glycogen of liver and muscle varies considerably (*Nutrition Reviews* **11,** *154 (1953)*); K. H. Meyer, *Advances in Enzymol.* **3,** *109 (1943)*). It is also known that glycogen is metabolically inhomogeneous within individual molecules, and that the turnover of glucosyl residues within the glycogen molecule is dependent upon at least two different types of enzymatic reactions which proceed at different rates (Marjorie R. Stetten and D. Stetten, *J. Biol. Chem.* **213,** *723 (1955)*). Whether the size of the molecules affects the rate of glycogen metabolism in normal liver and muscle is the question which arises.

This problem has been studied by M. R. Stetten, H. M. Katzen, and D. Stetten (*J. Biol. Chem.* **222,** *587 (1956)*) who used samples of glycogen which they isolated from liver and muscle of the rat and the rabbit. Precautions were taken to obtain glycogen with as little degradation during the isolation procedure as possible. This was done by extracting with cold trichloroacetic acid rather than by the conventional hot alkali method. By the use of molecular weight determinations and labeled glucose, it was found that liver and muscle glycogens were metabolized differently with respect to source and molecular weight. In liver the smaller molecules were more active metabolically than the larger molecules, whereas the larger molecules of muscle glycogen appeared to be more active metabolically.

No explanation for these differences in the metabolism of liver and muscle glycogen is apparent at present. The authors suggested that small differences in the action of muscle and liver phosphorylases may be involved. In any event, the studies point out that the turnover of glycogen cannot be described as the replacement of one finished glycogen molecule by another. In the accepted sense of the word there is no "finished" glycogen molecule.

These basic studies should aid in a clearer understanding of glycogen metabolism under normal conditions and in diabetes and other metabolic disorders of animals and man.

Recent Nutrition Book

Canadian Bulletin on Nutrition. (Recent Nutrition Surveys and Nutrition Education Programme in Cape Sable Island, Nova Scotia). Published by Department of National Health and Welfare, Ottawa, Canada, Vol. 4, No. 1, January, 1956. Pp. 54. Price: $.50. Copies of this publication may be obtained from the Department of Public Printing and Stationery, Ottawa, Canada.

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